Scientific Abstract

Erectile Dysfunction (ED) is defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual function. It has been estimated that 20 - 30 million men in the United States are affected.[1-4] A primary cause of ED in many impotent men is related to heightened contractility and/or impaired relaxation of corporal smooth muscle. More specifically, in the absence of a robust arterial and corporal smooth muscle relaxation response, the extant systemic blood pressure cannot be effectively transmitted to the penis, and thus, ED ensues. The use of Ion Channel Therapy for ED is based on the principle that corporal smooth muscle relaxation is dependent on a diminished intracellular calcium concentration. Smooth muscle cells that have been transfected with the a, or pore forming subunit of the human large conductance, calcium-sensitive K channel subtype, (*hSlo or Maxi-K*)**,** will express a greater number of physiologically relevant Maxi-K channels on the cell membrane. During neural stimulation, these additional Maxi-K channels are activated, leading to enhanced efflux of K^+ from the cells. The efflux of K^+ ions causes the cells to hyperpolarize, which in turn decreases the activity of the voltage-dependent calcium channels. As the activity of the Ca^{2+} channels is decreased, the influx of calcium ions will also decrease, resulting in decreased intracellular Ca^{2+} concentrations. The end result is that smooth muscle cell contraction is diminished and greater smooth muscle relaxation is achieved, allowing rigid erections to occur. In this scenario, the smooth muscle cells of the corpus cavernosum of *hSlo*-treated subjects have in essence been made "more sensitive" to neural relaxing stimuli, by virtue of their increased hyperpolarizing ability. It is anticipated that *hSlo* Ion Channel Gene Therapy will compensate for the diminished efficacy of the endogenous neural relaxing stimulus characteristic of organic erectile dysfunction in many impotent patients. All of our preclinical studies in the aged and experimentally diabetic rat model are consistent with this supposition. However, even in the worst-case scenario, it is envisioned that the "enhanced" relaxing ability of the smooth muscle cells will also permit other FDA-approved oral or intraurethral medications (i.e., Viagra or MUSE) to be even more efficacious, at lower doses, thus improving their safety profile.

The plasmid to be used consists of a 3-kb backbone, pVAX carrying a 3.9kb insert, hSlo cDNA, which codes for the pore forming subunit of the human max-K channel subtype. The pVAX (Invitrogen Inc.) vector is 2999 nucleotides in size. It contains the CMV promotor, T7 priming site; a multiple cloning site polylinker; Bovine Growth hormone reversible priming site; Bovine Growth hormone polyadenylation site; Kanamycin resistance marker and the pUC origin. This plasmid, therefore, contains both the gene encoding the human smooth muscle Maxi-K channel as well as the genetic machinery to control the transcription of the *hSlo* gene (CMV promoter, BGH PA). This "naked" DNA construct is referred to herein as *hMaxi-K.*

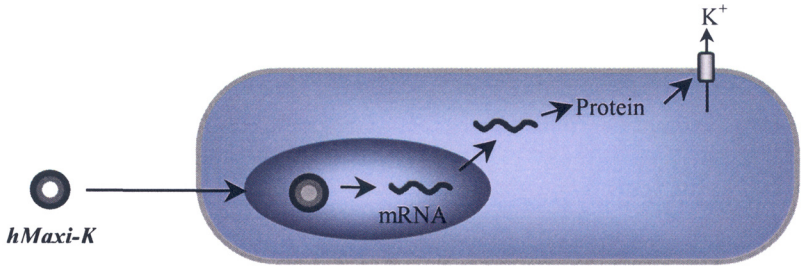

Figure 1. Illustration of a smooth muscle cell incorporating *hMaxi-K* plasmid into its nucleus, where transcription of the *hSlo* gene into a messenger RNA (mRNA) occurs. The transcribed mRNA then exits the nucleus through a nuclear pore and begins the translation process in the cytoplasm where protein synthesis for the Maxi-K channel occurs. The Maxi K channel is then incorporated into the cell membrane for regulation of potassium (K^+) efflux from the cell.

hMaxi-K ion channel therapy takes advantage of the high level of intercellular connectivity of corporal smooth muscle cells, such that only a small fraction of the smooth muscle cells need to be genetically modified to achieve global tissue effects. As such, *hMaxi-K* ion channel therapy works in synergy with these intercellular ion channels, known as gap junctions that are ubiquitously distributed among the smooth muscle cells of the penis (see Figure 2). Gap junctions are aqueous intercellular channels that provide partial cytoplasmic continuity between coupled cells, thus permitting the exchange of the ions (K^+, Ca^{2+}) and second messenger molecules (cAMP, cGMP, etc.,) that regulate smooth muscle contractility. In short, by using *hMaxi-K* to increase the expression of Maxi-K channels on only a small fraction of the smooth muscle cells, the enhanced hyperpolarizing currents so generated are readily transmitted to adjacent cells through the interconnecting gap junctions, ultimately resulting in a greater degree of smooth muscle relaxation for any given level of neural stimulation.

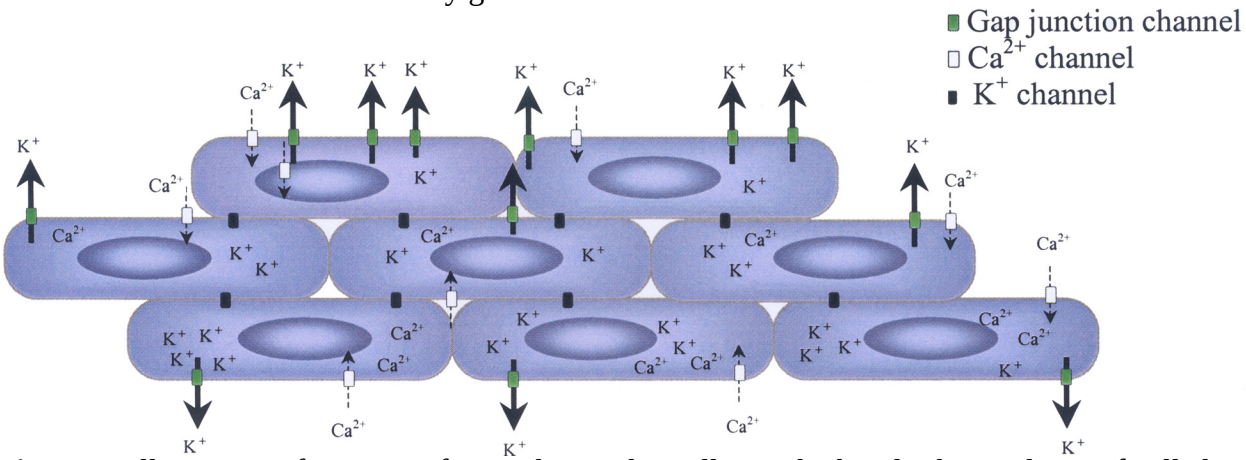

Figure 2. Illustration of a group of smooth muscles cells in which only the top layer of cells has been transfected with *hMaxi-K,* so that the Maxi-K channel is over-expressed on the surface of only these cells. In this arbitrary example of nonhomogeneous cellular transfection, activation of these novel Maxi K channels by neural stimulation leads to an augmented outflow of potassium ions (K^+) from the cells causing the cells to become hyperpolarized. Cellular hyperpolarization causes a decrease in membrane potential which in turn decreases the activity (i.e., the open probability or mean open time) of the voltage-dependent calcium channels. This parallel decrease in the activity of voltage-dependent Ca channels causes a decrease in the influx of

calcium ions (Ca^{++}), which corresponds to a decrease in intracellular Ca^{++} concentrations. Decreased intracellular calcium levels are associated with smooth muscle relaxation. This process is facilitated by the presence of gap junctions, which provide the means for the intercellular exchange of the ions and second messenger molecules required for the coordination of smooth muscle cell contraction and relaxation responses.

In essence, the gap junctions serve as the anatomic substrate for the signal amplification necessary for smooth muscle contraction and relaxation during neural stimulation. In the absence of gap junctions, more aggressive gene incorporation strategies (e.g. adenoviral or retroviral vectors) would need to be considered, thus increasing the risk of side effects. Such side effects include adverse immune responses and tissue inflammation caused by the viral vector. Inflammatory responses to the viral vector may actually inhibit the activity of the virus-based therapy and prohibit repeated administrations. Therefore, in the presence of gap junctions, non-viral gene therapy may offer the best opportunity to achieve the appropriate therapeutic response while minimizing adverse effects.

Preclinical studies in aged (retired breeder Sprague-Dawley rats) and experimentally diabetic rats, both with diminished erectile function, documented that treatment with a single intracavernous dose of Ion Channel Therapy results in restoration of normal penile rigidity measured by intracavernous pressure response to electrical stimulation of the cavernous nerve, and verified using a visual erectile scale. In fact, the responses of the *hSlo*-treated animals were similar to those of the young, normal or age-matched control animals. Moreover, the response in the *hSlo* -treated group was maintained for up to 6 months from a single injection.

In the proposed initial clinical study, the safety of a single intracavernous administration of *hSlo* plasmid will be evaluated. Additional assessments will include use of the International Index of Erectile Function questionnaire to evaluate the ability of subjects to engage in sexual intercourse with their partners and a Rigiscan device for measurement of visual sexual stimulation and sleep-induced erection.